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Classical pathway complement activation in association with paraproteinaemia

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Summary. Five of twenty-three patients with paraproteinaemia (two IgM, three IgG) have been shown to exhibit marked classical pathway complement activation. The mechanisms of hypocomplementaemia proposed for the five patients are cryoglobulinaemia in one and *in vivo* immunoglobulin aggregation in the other four. Three further patients had a low C1q and three a low C3 unassociated with any other complement abnormality. No association with any particular IgG subclass or obvious clinical abnormality existed in association with hypocomplementaemia.

INTRODUCTION

A number of abnormalities of the complement system have been observed in association with paraproteinaemia and/or a lymphoproliferative disorder (Kohler & Muller-Eberhard, 1969; Costanzi, Coltman & Donaldson, 1969; Glovsky & Fudenberg, 1970; Caldwell, Ruddy, Schur & Austen, 1972; Kohler & Muller-Eberhard, 1972; Jordan, McDuffie, Good & Day, 1974; Spitler, Spath, Petz, Cooper & Fudenberg, 1975; Schreiber, Zweiman, Atkins, Goldwein, Pietra, Atkinson & Abdon, 1976). Not all the mechanisms producing the observed complement abnormalities have been fully clarified, however.

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The present study was undertaken to examine in detail the classical pathway complement components in a group of patients with paraproteinaemia and to elucidate the causes of any abnormalities found.

MATERIALS AND METHODS

Patients

Twenty-three patients were included in the study. In all cases blood was taken for complement assays before treatment for paraproteinaemia had been started. The patients were unselected and represent almost consecutive cases diagnosed in the Department of Chemical Pathology.

Blood samples

15 ml blood were taken from all patients. 10 ml were clotted at room temperature for 1 h. 5 ml were taken in EDTA at a final concentration of 0·01 M EDTA in plasma (EDTA plasma). Serum and plasma were stored at $-70°$ until tested.

Paraproteins

Paraproteins were characterized by electrophoresis on cellulose acetate, and immuno-electrophoresis in agarose and were quantitated by radial immunodiffusion using Behringwerke Immunoplates.

7S IgM

The presence of 7S IgM was sought in the IgM paraprotein sera by Ouchterlony analysis in 4 per cent polyacrylamide gel (Caldwell *et al.*, 1972) against antihuman IgM (μ chain specific) (Behringwerke).

Complement component measurement

Immunoreactive C1q, C4 and C3 protein was measured by radial immunodiffusion using monospecific antisera (Behringwerke).

Functional assays for total haemolytic complement (CH50), C1 esterase inhibitor (C1 INH), C4 and C2 were performed by the methods of Lachmann, Hobart & Aston (1973), C1 was assayed by the use of EAC43 antrypol cells (Lachmann *et al.*, 1973) and a reagent deficient in C1 and C4 (R1.4) prepared from C4 deficient guinea-pig serum (Lachmann, personal communication).

Cryoglobulins

20–30 ml blood from those patients with evidence of complement activation were taken into syringes and transferred into glass bottles all warmed at 37°. Clotting was allowed to occur for $\frac{1}{2}$ h at 37°. After separation at 37°, the serum was incubated at 4° for 72 h. Any precipitate that formed was washed three times in complement fixation diluent (CFD) at 4° and redissolved in this buffer at 37°. Quantitation of immunoglobulins in the cryoprecipitate was undertaken using Behringwerke immunoplates incubated at 37°.

Evidence of complement activation

(a) *C1 and C4 activation.* The ability of paraprotein serum to activate C1 and/or C4 *in vitro* was assessed by incubation of test serum with pooled normal human serum (NHS) 1:1 either at 37° for 30 min or 4° for 1 h. C1 and C4 assays were carried out on the mixture after incubation as described above. Isolated cryoglobulins were also tested, at a concentration of 250 IU/ml.

(b) *C3 activation.* This was assessed by crossed immunoelectrophoresis using EDTA plasma (*in vivo* activation) or a 1:1 mixture of test serum and NHS previously incubated at 37° for 45 min (*in vitro* activation). EDTA at a final concentration of 0·01 M was added to the latter reaction mixture before electrophoresis.

Immunoglobulin aggregation

The ease with which immunoglobulins could be aggregated on heating was assessed. Serum was heated at 63° for 2 min, then incubated with NHS 1:1 at 37° for 30 min and the resulting C1 titre was assayed. The results were expressed as a percentage fall of C1 activity corrected for the fall in C1 in NHS that resulted from 2 min incubation at 63°. 2 min incubation time was chosen because longer incubation induced marked C1 activation in NHS alone.

Immunoglobulin to albumin ratio

This was expressed as the ratio of total immunoglobulin (expressed as g/l) to albumin. The latter was measured by a routine autoanalyser technique.

RESULTS

Paraprotein (Table 1)

Nine patients had an IgG paraprotein, seven an IgA, five an IgM and one an IgD. One patient had circulating free kappa light chains.

7S IgM (Table 1)

7S IgM was demonstrated in three IgM paraproteinaemic sera.

Complement components (Table 1)

Five patients were found to have low levels of C1 and C4 (these patients are referred to subsequently as hypocomplementaemic), three of whom also had low C2; three had low C1q. One patient had a low C1 INH level. All had normal C3. The C4 levels in EDTA plasma were no different from those in serum.

Isolated low levels of various components were present in a total of eight other patients.

Cryoglobulinaemia

Two hypocomplementaemic sera contained detectable cryoglobulins. One consisted of IgG alone at a concentration of 670 IU/ml in serum and one IgG and IgM at concentrations of 52 IU/ml and 274 IU/ml respectively.

Complement activation

No evidence of *in vivo* C3 activation was found in any plasma. *In vitro* C3 conversion occurred with the serum of one hypocomplementaemic patient and with both isolated cryoglobulins.

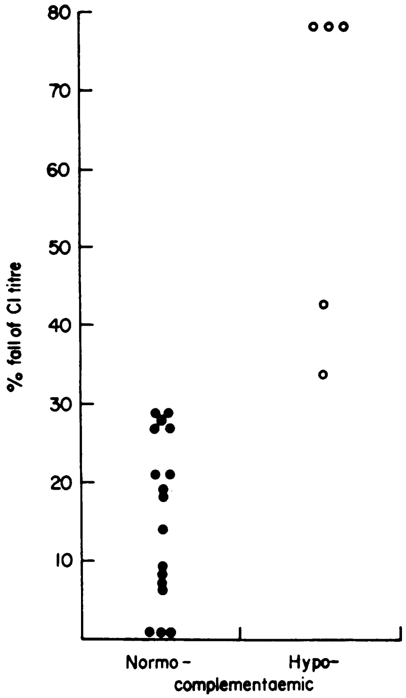

Figure 1

***In vitro* C1 or C4 activation**

No evidence of C1 or C4 activation either at 4° or 37° was found with four hypocomplementaemic sera or with the isolated IgG/IgM cryoglobulin. The serum containing IgG cryoglobulin and the isolated cryoglobulin caused complete C1 and C4 activation immediately on mixing with NHS.

Evidence of heat induced immunoglobulin aggregation (Fig. 1)

Some activation of C1 was evident on incubation of heated NHS with NHS as it was with those sera in which there was no evidence of complement activation *in vivo*. However, C1 activation was markedly greater after incubation with heated serum from the five hypocomplementaemic patients.

Immunoglobulin to albumin ratio (Table 1)

The hypocomplementaemic sera did not show a higher ratio than the normocomplementaemic group.

Relationship of complement activation to subclass of IgG paraprotein (Table 2)

No obvious differences in IgG subclasses existed between hypocomplementaemic and normocomplementaemic IgG paraprotein sera.

DISCUSSION

Five out of twenty-three patients with paraproteinaemia (21·7 %) have been shown to have evidence of marked classical pathway complement activation, indicated by low levels of C1, C4 and C2. We have presented evidence for two possible mechanisms of complement activation, those of cryoglobulinaemia and paraprotein aggregation. One serum was found to contain an IgG cryoglobulin which caused *in vitro* activation of C1, C4 and C3. It is probable that these events also occurred *in vivo*. An IgG/IgM cryoglobulin which was detected in one other serum also cause *in vitro* activation of C3 but not of C1 or C4. However, both this latter serum and the other three hypocomplementaemic sera caused greater *in vitro* C1 activation after heating at 63° for 2 min than normal human serum or normocomplementaemic paraprotein containing sera. It is possible, therefore, that *in vivo* paraprotein aggregation might occur with greater ease in these patients than in normal subjects and result in complement activation. If such aggregates were cleared quickly from the circulation, it might explain the absence of *in vitro* C1, C4 and C3 activation when unheated test serum and NHS were incubated together. It is apparent too that a preponderance of IgG subclasses known to activate complement did not exist in the hypocomplementaemic IgG paraprotein sera. This further supports our proposal that a physicochemical abnormality of paraprotein existed in the hypocomplementaemic sera resulting in cryoprecipitability or ease of immunoglobulin aggregation. Glovsky & Fudenberg (1970) described eleven patients with Waldenstrom's macroglobulinaemia up to half of whom had low levels of C1, C2 or C3, but were unable to define the mechanism(s) of hypocomplementaemia. However, differences of electrophoretic mobility of the IgM paraproteins associated with complement abnormalities were shown, which might indicate, as we propose, that differences of immunoglobulin structure or function may be partly

Table 1. Complement component and immunoglobulin levels. Normal values at head of each column. Complement components (H = haemolytic, I = immunochemical) expressed as percentage pooled normal human serum, immunoglobulins as international units per millilitre. Italic immunoglobulin figures indicates paraprotein. C4 figures in parentheses indicate level in EDTA plasma

	30–190 CH50	45–210 C1H	70–150 C1qI	⩾70 C1INH	50–210 C4 I	75–125 C4 H	60–140 C2 H	70–120 C3 I	80–220 IgG	50–270 IgA	50–250 IgM	Immunoglobulin /albumin ratio
1	58	132	104	77	102	150	110	100	82	*1300*	22	0·6
2	62	82	92	108	100	74	130	55	26	10	*21,000*¶	9·6
3	74	92	107	77	120	150	108	90	*475*	37	98	1·2
4	68	77	33	116	314	74	90	104	49	*2940*	42	2·7
5		82	85	79	50	110	90	117	*454*	134	68	1·2
6	64	160	150	75	66	110	67	70	185	89	*8350*	1·8
7	64	85	190	115	129	120	63	120	*1310*	< 20	28	6·7
8	106	120	180	115	162	33	100	139	90	*780*	42	0·5
9*	86	240	100	131	231	120	100	129	63	32	96	—
10	68	85	100	88	77	100	100	67	114	*760*	84	0·7
11†	94	100	100	100	121	110	110	128	49	< 20	< 20	0·1
12		74	90	100	96	120	64	90	41	< 20	*600*¶	0·3
13	86	53	100	81	126	100	75	132	92	*380*	40	0·2
14	58	90	52	94	108	100	100	81	58	*3400*	43	1·7
15	196	90	100	106	131	47	170	124	*174*	288	94	0·4
16	106	90	100	117	191	100	100	132	*172*	152	102	0·4
17	112	33	105	100	53	110	130	148	*885*	22	40	2·3
18	46	66	47	90	111	90	120	55	37	*3700*	22	1·9
19	< 10	0	33	32	0(0)	0	15	113	108	24	*2020*	0·7
20‡	< 10	33	68	100	12(11)	0	25	80	82	62	*970*¶	0·2
21§	< 10	0	130	80	44(42)	0	57	174	*460*	22	156	1·1
22	< 10	26	47	85	17(24)	24	82	138	*732*	27	34	1·6
23	< 10	33	100	85	13(10)	17	82	166	*304*	24	163	0·9

* Kappa light chains; † IgD; ‡ IgG/IgM cryoglobulin; § IgG cryoglobulin; ¶ 7S IgM present.

or wholly the cause. There was no evidence that a higher immunoglobulin to albumin ratio existed in the hypocomplementaemic group to explain the ease of *in vitro* heat induced aggregation. It is perhaps significant that none of the paraproteins in our hypocomplementaemic sera were IgA, as IgA does not activate the classical pathway (Spiegelberg, 1974). It is unlikely that the low C1, C4 and C2 levels in this group were due to complement activation during *in vitro* coagulation (Glovsky & Alenty, 1973) as the C4 levels in EDTA were insignificantly different to those in serum. It is probable that the single low C1 INH was secondary to activation of the classical pathway, rather than a primary deficiency, as C1 was

Table 2. IgG subclass concentrations in IgG paraprotein sera (normal values at head of each column)

IgG 1 370–1230 mg/100 ml	IgG 2 46–420 mg/100 ml	IgG 3 15–245 mg/100 ml	IgG 4 1·5–18·5 mg/100 ml
3920*	1986	25	Trace
2298*	1146	104	Trace
1048*	1986	201	49
879	443	39	164
845	443	80	Trace
1217	520	188	49
1927	520	107	139
2028	2521	118	49
554	3820	94	21

* Hypocomplementaemic sera.

low and levels of C1q, C4 and C2 were the lowest of the hypocomplementaemic group. 7S IgM was not demonstrated in this serum, although it was present in two normocomplementaemic and one hypocomplementaemic sera, all of which had normal C1 INH levels.

Eight other patients showed low levels of complement components, three had a low C1q, and it is possible that these patients are similar to those in whom hypercatabolism and extravascular sequestrian of C1q occurs as described by Kohler & Muller-Eberhard (1972). Interestingly, all three patients had an IgA paraproteinaemia as did a total of three patients studied by Kohler & Muller-Eberhard (1969, 1972). Two patients had a low level of haemolytic C4 and one other had a low haemolytic C1. In the absence of any other component abnormalities and normal levels of immunochemical C4 and C1q respectively these results may reflect ageing of the sera, rather than *in vivo* complement activation. Three patients had a low C3 alone, as did three of Glovsky & Fudenberg's (1970) cases. No explanation of this finding is apparent; certainly no evidence of *in vitro* C3 converting activity was present in their sera.

In conclusion, we propose that the marked *in vivo* complement activation present in five out of twenty-three patients with paraproteinaemia may be related to small degrees of *in vivo* immunoglobulin aggregation in four and to cryoglobulinaemia in the other. These results may indicate that complement activation in association with paraproteinaemia is commoner than has been reported previously although there were no obvious clinical abnormalities associated with these findings.

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